Bull. Pharm. Sci., Assiut University, Vol. 45, Issue 1, 2022, pp. 451-458.

Bulletin of Pharmaceutical Sciences
Assiut University
Website: http://bpsa.journals.ekb.eg/
e-mail: bullpharm@aun.edu.eg

INVESTIGATING BACTERIA ISOLATED FROM DIABETIC FOOT ULCERS AND STUDYING THEIR SENSITIVITY TO ANTIBIOTICS – SYRIA

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The aim of this study was to investigate bacteria isolated from diabetic foot ulcers (DFUs), and determine their antibiotic sensitivity patterns that helps clinicians to select appropriate antimicrobial therapy. The study included 65 patients with DFUs who were admitted to Al-Basel Hospital in Homs, Syria between May 2020 and December 2020. Bacteria were isolated from foot lesions and identified by colonial morphology, gram staining and biochemical reactions. Antibiotic sensitivity of isolates was determined using the Kirby-Bauer disk diffusion method. A total of 89 bacterial isolates were obtained from 63 patients. Gram-positive bacteria were more common (58%) than gram-negative bacteria (42%). Staphylococcus aureus was the most prevalent isolate (29%), followed by Pseudomonas aeruginosa (13%) and Streptococcus agalactiae (11%). The antibiotic imipenem was the most effective against both gram-positive and gram-negative bacteria. In addition to imipenem, vancomycin and linezolid were the most effective antibiotics against gram-positive bacteria, while gentamicin and amikacin were the most effective antibiotics against gram-negative bacteria. This study showed low levels of sensitivity to self-administered antibiotics. Therefore, there is a need to avoid excessive use of antibiotics and improve antimicrobial stewardship programs.

Keywords: *diabetic foot ulcers, sensitivity patterns, antimicrobial stewardship.*

INTRODUCTION

Diabetes mellitus (DM) affects more than 460 million numerous of individuals globally[1].

Diabetics have a 12% to 25% lifetime risk of developing diabetic foot ulcers (DFUs)[2], usually because of diabetes-associated peripheral neuropathy, peripheral arterial disease, and foot deformity[3]. Diabetic foot infections (DFIs) were considered as one of the most commonly and catastrophic complications of diabetes[4], which are associated with increased hospitalizations, worsening outcomes, and increased amputation rates[5-6].

The bacteriology of DFIs is generally polymicrobial[2-7]. However, many studies have been conducted on the microbial etiology of DFIs with disputed results due to many differences such as geographical regions, type of infections or method used in bacterial culture[8-9].

The treatment of DFIs, like other infections, is becoming increasingly difficult due to the massive consumption of antibiotics, which is largely responsible for emerging antimicrobial resistance[10].

The appropriate selection of antibiotics in DFIs management is based on knowledge about causative organisms, and their sensitivity patterns. Therefore, the aim of the present study was to investigate bacteria isolated from DFUs and determine their sensitivity patterns to a variety of commonly used antibiotics that helps clinicians to select appropriate antimicrobial therapy.

Received in 02/1/2022 & Accepted in 28/1/2022

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MATERIALS AND METHODS

Patients

This study included 65 diabetic patients with DFUs, who were admitted to Al-Basel Hospital in Homs, Syria between May 2020 and December 2020. The patients underwent debridement of their DFUs, and all of them were taking antibiotics. Demographic and clinical features including age, sex, type and duration of diabetes, complications of diabetes and antibiotics therapy prescription were gathered for each patient.

Specimen Collection and Microbiological Culturing

Swab samples were collected from each ulcer after the ulcer had been cleansed by 0.9% sterile saline and gauze[11]. Each lesion was swabbed by sterile swab, which was rotated over a 1 cm^2 area of the lesion for five seconds, using sufficient pressure to get fluid from the deeper portion of the ulcer[12]. The specimens were placed into sterile transport tubes and sent immediately to the microbiology laboratory at the Faculty of pharmacy, Al-Baath University for aerobic culturing. Specimens were inoculated onto agar plates (blood agar (5% sheep blood), chocolate agar (boiled blood agar), MacConkey agar) and thioglycollate broth. The inoculated plates and broth were incubated at 37 °C overnight[13]. Traditional methods (colonial morphology, gram staining and biochemical reactions) were used to identify the microorganisms[14].

Antibiotic Sensitivity Testing

The Kirby Bauer disk diffusion test (the disk-diffusion method) was used according to standard CLSI protocols[15]. The antibiotics tested were amoxicillin/clavulanic acid 30/10 µg, gentamicin 30µg, amikacin 30µg, ciprofloxacin 5µg, ofloxacin 10µg, levofloxacin 5µg, norfloxacin 10µg, vancomycin 30µg, linezolid 30µg, erythromycin 15µg, azithromycin 15µg, clarithromycin 15µg, cefotaxime 30µg, cefadroxil 30µg, cefepime 30 µ g, ceftriaxone 30µg,fucidic acid 10µg, trimethoprim + sulfamethoxazole 25µg, imipenem 10 µg.

RESULTS AND DISCUSSION

Results

Characteristic of patients

The present study included 65 diabetic patients, and of these patients, 41 (63%) were male and 24 (37%) were female. The mean age of the patients was 61.7 ± 10.33 years (mean ± SD; range, 35-75 years). All the patients enrolled were type 2 diabetes ones.

The duration of diabetes was ≤ 5 years in 8 (12.30%) patients, 6-10 years in 14 (21.53%) patients, and > 10 years in 43 (66.15%) patients.

Regard to complications of diabetes, there were 27 (41.53%) patients with vasculopathy, 15 (23.07%) patients with hypertension, 35 (53.84%) patients with neuropathy, 11 (16.92%) patients with nephropathy, 6 (9.23%) patients with retinopathy. Additionally, all the patients in our study were taking antibiotics in their own home by self-administration.

The Demographic and clinical data of diabetic foot patients have been summarized in Table 1.

Bacterial examination

Among the 65 study patients, the specimens were culture-positive in 63 (97%) and were negative in the remaining 2 (3%) patients. A total of 89 bacterial isolates were obtained from the 63 patients in whom the specimens were culture-positive. In this study, gram-positive bacteria represented 58% (n= 52) of the isolates, and gram-negative bacteria represented 42% (n= 37).

The bacteria that were isolated from the DFUs are summarized in Table 2. *Staphylococcus aureus* [26 (29%) isolates] was the most commonly isolated bacteria among the gram-positive bacteria, followed by *Streptococcus agalactiae* [10 (11%) isolates], *Enterococcus faecalis* [6 (7%) isolates], *Staphylococcus epidermidis* [5 (6%) isolates], and *Staphylococcus saprophyticus* [5 (6%) isolates]. On the other hand, *Pseudomonas aeruginosa* [12 (13%) isolates] was the main gram-negative bacteria followed by *Klebsiella pneumonia* [7 (8%) isolates], *Escherichia coli* [5 (6%) isolates], *Proteus mirabilis* [5 (6%) isolates], *Enterobacter* spp. [4 (4%) isolates], and *Acinetobacter baumannii* [4 (4%) isolates].

Table 1 : Demographic and clinical data of diabetic foot patients

parameter	Value (n=65)	
	n	%
Age, years	61.7 ± 10.33	
Sex		
Male	41	63
Female	24	37
Diabetic type		
Type 1	0	0
Type 2	65	100
The duration of diabetes mellitus		
≤5 years	8	12.30
6-10 years	14	21.53
>10 years	43	66.15
Complication		
Vasculopathy	27	41.53
Hypertension	15	23.07
Neuropathy	35	53.84
Nephropathy	11	16.92
Retinopathy	6	9.23
Antibiotics therapy prescription		
Self-administered	65	100
By physicians	0	0

Data are presented as mean ± standard deviation or number (percentage)

Table 2 : Bacteria isolated from the diabetic ulcers

Bacteria isolated	Value (n=89)	
	n	%
Staphylococcus aureus	26	29
Streptococcus agalactiae	10	11
Enterococcus faecalis	6	7
Staphylococcus epidermidis	5	6
Staphylococcus saprophyticus	5	6
Pseudomonas aeruginosa	12	13
Klebsiella pneumonia	7	8
Escherichia coli	5	6
Proteus mirabilis	5	6
Enterobacter spp.	4	4
Acinetobacter baumannii	4	4

Antibiotic sensitivity patterns of the isolates

The antibiotic sensitivity patterns of the isolates are summarized in Table 3 and Table 4. It was found that imipenem was the most effective antibiotic against *Staphylococcus aureus* (100%), *Streptococcus agalactiae* (100%), *Enterococcus faecalis* (100%), *Staphylococcus epidermidis* (100%) *and Staphylococcus saprophyticus* (100%). Additionally, *Staphylococcus aureus* was sensitive to linezolid (96.15%) and vancomycin (92.30%). All strains of *Streptococcus agalactiae*, *Enterococcus faecalis*, *Staphylococcus epidermidis* and *Staphylococcus saprophyticus* were sensitive to linezolid and vancomycin.

Pseudomonas aeruginosa was sensitive to imipenem (100%), gentamicin (83.33%) and amikacin (75%). *Klebsiella pneumonia* was sensitive to imipenem (100%), ciprofloxacin (71.42%) and norfloxacin (71.42%). *Escherichia coli* was sensitive to gentamicin (100%), amikacin (100%) and imipenem (100%). *Proteus mirabilis* was sensitive to amikacin (100%), imipenem (100%) and gentamicin (80%). *Enterobacter* spp. were sensitive to gentamicin (100%), amikacin (100%), cefepime (100%), ceftriaxone (100%) and imipenem (100%). *Acinetobacter baumannii* was only sensitive to imipenem (100%) and trimethoprim+sulfamethoxazole (50%).

Table 3 : Antibiotic sensitivity patterns of 52 Gram-positive bacteria

Antibiotic	***Staphylococcus aureus* (n=26)**		***Streptococcus agalactiae* (n=10)**		***Enterococcus faecalis* (n=6)**		***Staphylococcus epidermidis* (n=5)**		***Staphylococcus saprophyticus* (n=5)**	
	n	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	%
Amoxi+Clavulanic	19	73.07	5	50	5	83.33	3	60	2	40
Gentamicin	17	65.38	3	30	4	66.66	3	60	2	40
Amikacin	19	73.07	7	70	4	66.66	3	60	5	100
Ciprofloxacin	18	69.23	5	50	6	100	3	60	2	40
Ofloxacin	20	76.92	5	50	0	0	3	60	2	40
Levofloxacin	18	69.23	7	70	6	100	3	60	2	40
Norfloxacin	19	73.07	5	50	5	83.33	2	40	2	40
Vancomycin	24	92.30	10	100	6	100	5	100	5	100
Linezolid	25	96.15	10	100	6	100	5	100	5	100
Erythromycin	9	34.61	3	30	0	0	2	40	2	40
Azithromycin	9	34.61	3	30	0	0	2	40	2	40
Clarithromycin	11	42.30	3	30	0	0	3	60	5	100
Cefotaxime	10	38.46	3	30	ND		3	60	2	40
Cefadroxil	14	53.84	3	30	ND		3	60	2	40
Cefepime	12	46.15	3	30	ND		3	60	2	40
Ceftriaxone	13	50	3	30	ND		3	60	2	40
Fucidic acid	17	65.38	ND		ND		0	0	0	0
Trimethoprim+ Sulphamethoxazole	19	73.07	3	30	ND		2	40	2	40
Imipenem	26	100	10	100	6	100	5	100	5	100

ND: not detected

Table 4: Antibiotic sensitivity patterns of 37 Gram-negative bacteria

Antibiotic	***Pseudomonas aeruginosa* (n=12)**		***Klebsiella pneumonia* (n=7)**		***Escherichia coli* (n=5)**		***Proteus mirabilis* (n=5)**		***Enterobacter* spp. (n=4)**		***Acinetobacter baumannii* (n=4)**	
	n	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	%
Amoxi+ Clavulanic	1	8.33	1	14.28	2	40	0	0	0	0	0	0
Gentamicin	10	83.33	3	42.85	5	100	4	80	4	100	0	0
Amikacin	9	75	4	57.14	5	100	5	100	4	100	0	0
Ciprofloxacin	8	66.66	5	71.42	3	60	3	60	1	25	0	0
Ofloxacin	9	75	3	42.85	3	60	3	60	1	25	0	0
Levofloxacin	7	58.33	4	57.14	3	60	3	60	1	25	0	0
Norfloxacin	8	66.66	5	71.42	3	60	3	60	1	25	0	0
Vancomycin	ND		ND		ND		ND		ND		ND	
Linezolid	ND		ND		ND		ND		ND		ND	
Erythromycin	ND		ND		ND		ND		ND		ND	
Azithromycin	ND		ND		ND		ND		ND		ND	
Clarithromycin	ND		ND		ND		ND		ND		ND	
Cefotaxime	4	33.33	2	28.57	1	20	2	40	3	75	0	0
Cefadroxil	4	33.33	2	28.57	1	20	2	40	3	75	0	0
Cefepime	4	33.33	2	28.57	1	20	2	40	4	100	0	0
Ceftriaxone	4	33.33	4	57.14	2	40	3	60	4	100	0	0
Fucidic acid	ND		ND		ND		ND		ND		ND	
Trimethoprim+ Sulphamethoxazole	1	8.33	1	14.28	1	20	3	60	0	0	2	50
Imipenem	12	100	7	100	5	100	5	100	4	100	4	100

ND: not detected

Discussion

In the present study, we found that majority of patients with DFUs were male and over 60 years old, which are in agreement with other studies that described older age[16] and male sex[17]as demographic risk factors of DFUs. Regard to the clinical findings of the current study, similar to a previous one[18], type 2 diabetes with duration more than 10 years and neuropathy were other factors associated with the risk of DFUs among the study patients.

A total of 89 bacterial isolates were obtained from 63 patients. Gram-positive bacteria were the most common. This result is in agreement with many studies[16-19], but other works[13-20] reported gram‑ negative isolates as the most prevalent aerobic infection in DFUs.

As the other studies[9-21], *Staphylococcus aureus* was the main causative pathogen in DFIs followed by *Pseudomonas aeruginosa.* In addition, *Streptococcus agalactiae* ranked third among the isolates.

In patients with DFIs, the association of antibiotic resistance with the inappropriate use of antibiotics was described[22]. All antibiotic therapies in our study were self-administered by the patients due to weakness in antimicrobial stewardship activities locally.

In our study, like many others[16], imipenem was found to be the most effective antibiotic against Staphylococcus aureus. Other antibiotics such as vancomycin and linezolid were also highly effective for gram‑ positive coverage. Among gram-negative bacteria, Pseudomonas aeruginosa and the Enterobacteriaceae family (Klebsiella pneumonia, Escherichia coli, Proteus mirabilis, Enterobacter spp.) showed the highest sensitivity to imipenem among the tested antibiotics, which is consistent with a previous work[23]. In addition to imipenem, gentamicin and amikacin were also sensitive for the majority of gram-negative bacteria.

The present study like few others[21] noted low levels of sensitivity to macrolides and cephalosporins among gram positive-bacteria. Amoxicillin/clavulanic acid and trimethoprim/sulfamethoxazole, similar to a previous report[19], were the least effective against gram-negative bacteria.

Acinetobacter baumannii, which displays successful ability to acquire antimicrobial resistance[24]**,** was the most bacteria showing a very low degree of sensitivity to almost all the tested antibiotics.

The low rates of sensitivity to antibiotics, such as β-Lactams, fluoroquinolones, and macrolides, shown in our study may be attributed to the fact that these antibiotics are freely available for purchase without a medical prescription, for this reason they had been widely abused and frequently implicated in self-medication other than some antibiotics, which are prescribed in hospitals and under strict medical supervision, such as imipenem and linezolid.

Conclusions

We provided an updated picture of the bacterial profile and antibiotic sensitivity patterns of isolated bacteria in DFUs. The findings of this study indicate that Staphylococcus aureus was the most commonly bacteria followed by Pseudomonas aeruginosa and Streptococcus agalactiae. Highest sensitivity of gram-positive bacteria was seen with imipenem, vancomycin and linezolid. While imipenem, gentamicin and amikacin were the three most effective drugs against gram-negative bacteria. We noted low levels of sensitivity to self-administered antibiotics. Therefore, there is a need to avoid excessive use of antibiotics and improve antimicrobial stewardship activities that may can help in the future.

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Bull. Pharm. Sci., Assiut University, Vol. 45, Issue 1, 2022, pp. 451-458.

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تحرّي الجراثيم المعزولة من قرحات القدم السكريّة ودراسة حساسيّتها للصّادّات الحيويّة – سوريا

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كان الهدف من هذه الدّراسة هو تحرّي الجراثيم المعزولة من قرحات القدم السكريّة (DFUs) وتحديد أنماط حساسيّتها للصادّات الحيويّة الذي يساعد الأطباء في اختيار العلاج المناسب بمضادّات الميكروبات. اشتملت الدّراسة على ٦٥ مريض مصابين بقرحات قدم سكريّة الذين تمّ قبولهم في مستشفى الباسل في حمص، سوريا بين أيّار ٢٠٢٠ وكانون الأول ٢٠٢٠. تمّ عزل الجراثيم من آفات القدم وتمّ التعرّف عليها من خلال شكل المستعمرات، تلوين غرام والاختبارات الكيميائيّة الحيويّة. تمّ تحديد حساسيّة العزلات للصّادّات الحيويّة بطريقة انتشار القرص كيربي باور. تمّ الحصول على ٨٩ عزلة جرثوميّة من ٦٣ مريض. كانت الجراثيم إيجابيّة الغرام أكثر شيوعاً (٥٨%) من الجراثيم سلبيّة الغرام (٤٢%). كانت المكوّرات العنقوديّة المذهّبة هي العزلة الأكثر انتشاراً (٢٩%)، تليها الزّوائف الزنجاريّة (١٣%) والمكوّرات العقديّة القاطعة للدرّ (١١%). كان الصّاد الحيوي إيميبينيم الأكثر فاعليّة ضدّ كلّ من الجراثيم إيجابيّة الغرام وسلبيّة الغرام. بالإضافة إلى الإيميبينيم، كان الفانكومايسين واللينزوليد الأكثر فاعليّة ضد الجراثيم إيجابيّة الغرام، بينما كان الجنتاميسين والأميكاسين أكثر الصّادات الحيويّة فاعليّة ضدّ الجراثيم سلبيّة الغرام. أظهرت هذه الدّراسة مستويات منخفضة من الحساسيّة للصّادات الحيويّة التي يتم تناولها ذاتيّاً. وبالتّالي، هناك حاجة لتجنّب الاستخدام المُفرط للصّادات الحيويّة وتحسين برامج الإشراف على مضادّات الميكروبات.